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## Population dynamics of methicillin-susceptible and -resistant *Staphylococcus aureus* in remote communities

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**Objectives:** Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) was first reported in remote regions of Western Australia (WA) in 1992 and is now the predominant MRSA isolated in the State. To gain insights into the emergence of CA-MRSA, 2146 people living in 11 remote WA communities were screened for colonization with *S. aureus*.

**Methods:** Antibigram analysis, contour-clamped homogeneous electric field electrophoresis, multi-locus sequence typing, Pantón–Valentine leucocidin determinant detection and accessory genetic regulator typing were performed to characterize the isolates. MRSA was further characterized by staphylococcal cassette chromosome *mec* typing.

**Results:** The *S. aureus* population consisted of 13 clonal complexes and two Singleton lineages together with 56 sporadic isolates. Five lineages contained MRSA; however, these were not the predominant methicillin-susceptible *S. aureus* (MSSA) lineages. There was greater diversity amongst the MSSA while the MRSA appeared to have emerged clonally following acquisition of the staphylococcal cassette chromosome *mec*. Three MRSA lineages were considered to have been endemic in the communities and have subsequently become predominant lineages of CA-MRSA in the wider WA community. People colonized with MSSA tended to harbour clones of a different genetic lineage at each anatomical site while people colonized with MRSA tended to harbour clones of the same lineage at each site. Overall, the isolates were resistant to few antimicrobials.

**Conclusions:** Although the evidence suggests that in WA CA-MRSA strains arose in remote communities and have now disseminated into the wider community, there is no evidence that they arose from the predominant MSSA clones in these communities.

**Keywords:** *S. aureus*, community methicillin-resistant *Staphylococcus aureus*, population structure, colonization

### Introduction

*Staphylococcus aureus* is one of the most successful pandemic bacterial pathogens. It is also a ubiquitous inhabitant of human microbiological flora, with up to 30% of humans persistently colonized asymptomatically, and up to 70% intermittently colonized.<sup>1</sup>

Initially MRSA was found almost exclusively in hospitals where it became known as healthcare-associated MRSA (HA-MRSA). However, it has now emerged in communities around the world where it is known as community-associated MRSA (CA-MRSA). The earliest reports of CA-MRSA involved infections in people from isolated Indigenous<sup>2</sup> or disadvantaged

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communities,<sup>3</sup> suggesting that these were the primary environments from which it emerged. In Australia the first CA-MRSA, colloquially known as 'WA MRSA', was reported in 1993 in infected Indigenous people from remote communities in the sparsely populated Kimberley region of Western Australia (WA).<sup>2</sup> This was followed by reports of CA-MRSA from Indigenous people in the Northern Territory,<sup>4</sup> Queensland<sup>5</sup> and Central Australia.<sup>6</sup>

CA-MRSA is responsible for a wide spectrum of infections, from uncomplicated skin and soft tissue infections through to necrotizing pneumonia, necrotizing fasciitis and bacteraemia, which can be fatal in otherwise healthy people. This virulence has been attributed to the possession of virulence determinants, such as the Panton–Valentine leucocidin (PVL).<sup>7</sup> Apart from isolated instances, CA-MRSA was resistant to few non- $\beta$ -lactam antibiotics and did not initially spread in hospitals. However, the epidemiology of CA-MRSA is changing and multiply resistant CA-MRSA spreading in hospitals, communities and internationally has been reported.<sup>8,9</sup>

To prevent the transmission of MRSA in WA hospitals, MRSA was made a notifiable organism and a 'search and destroy' policy was introduced in 1982. As part of this policy all isolates are sent to a reference centre for typing and storage.<sup>10</sup> Although this strategy has not prevented the spread of CA-MRSA, which now comprises 77.5% of MRSA isolated in WA,<sup>11</sup> it has enabled its spread to be closely monitored. Surveillance data have shown that between 1983 and 2002 the notification rates for CA-MRSA in WA increased >50- and 70-fold in rural and metropolitan health regions, respectively.<sup>12,13</sup>

CA-MRSA utilizes mobile elements and single nucleotide polymorphisms to establish local and geographic niches<sup>14</sup> and is thought to emerge when a locally prevalent strain of methicillin-susceptible *S. aureus* (MSSA) acquires a staphylococcal cassette chromosome *mec* (SCC*mec*) element. The remote WA Indigenous communities provide an ideal environment in which to study the natural genetics of *S. aureus* and CA-MRSA as the population has limited contact with healthcare institutions and therefore HA-MRSA. Consequently, surveys of populations from remote WA communities were undertaken between 1995 and 2003. The aims of this study were to determine the colonization dynamics and genetics of *S. aureus* in the communities and to gain insights into the emergence of CA-MRSA.

## Materials and methods

Ethics approval for the screening of Indigenous communities was obtained from the WA Aboriginal Health Information and Ethics Committee and the Curtin University of Technology Human Research Ethics Committee. Prior to each survey a senior member of the team travelled to each community to obtain permission from the community Elders and Councils. Remote region healthcare professionals and Indigenous aides provided valuable support. Although participation in the survey was voluntary, on most occasions participation was near to 100%. Written informed consent was obtained from each adult individual, parent or guardian.

### Communities

The inhabitants of 11 major remote communities from three geographical regions of WA, the Kimberley, the Pilbara and the Goldfields, were screened for *S. aureus* colonization (Figure 1). The

community population sizes were between 60 and 400 people. Small fringe or satellite communities with populations of between 9 and 51 were also screened and their results were combined with results for the larger community in their geographical proximity. The communities were 700–2000 km from the capital city Perth and their geographical regions accounted for 6.4% of the total WA population. While for each episode of community screening inhabitants were screened only once, it was not possible to determine the number of times an individual was screened over the 9 year duration of the surveys due to ethical constraints and the nomadic nature of the population. Therefore, each screening episode has been enumerated as a set of screening swabs only.

### Screening

Overall, 2146 sets of screening swabs were collected; 924 from three Kimberley communities, 258 from a Pilbara community and 964 from seven Goldfields communities. Community 2 was screened in June (dry season) and December (wet season) of 1995, communities 3 and 4 were screened in 1995, 1999 and 2003, and community 7 was screened in 1999 and 2003. The remaining communities, 1, 5, 6, 8, 9, 10 and 11, were screened once in 1995, 1996, 1998, 1999, 2001, 2001 and 2001, respectively (Figure 1).

The anterior nares, throat and, when applicable, up to two skin lesions were swabbed with moistened cotton wool swabs. Swabs were placed in Amies transport medium (Interpath services, Pty Ltd, West Heidelberg, Australia) and transported in insulated containers by road and air to the laboratory in Perth. All swabs were processed within 48 h of collection.

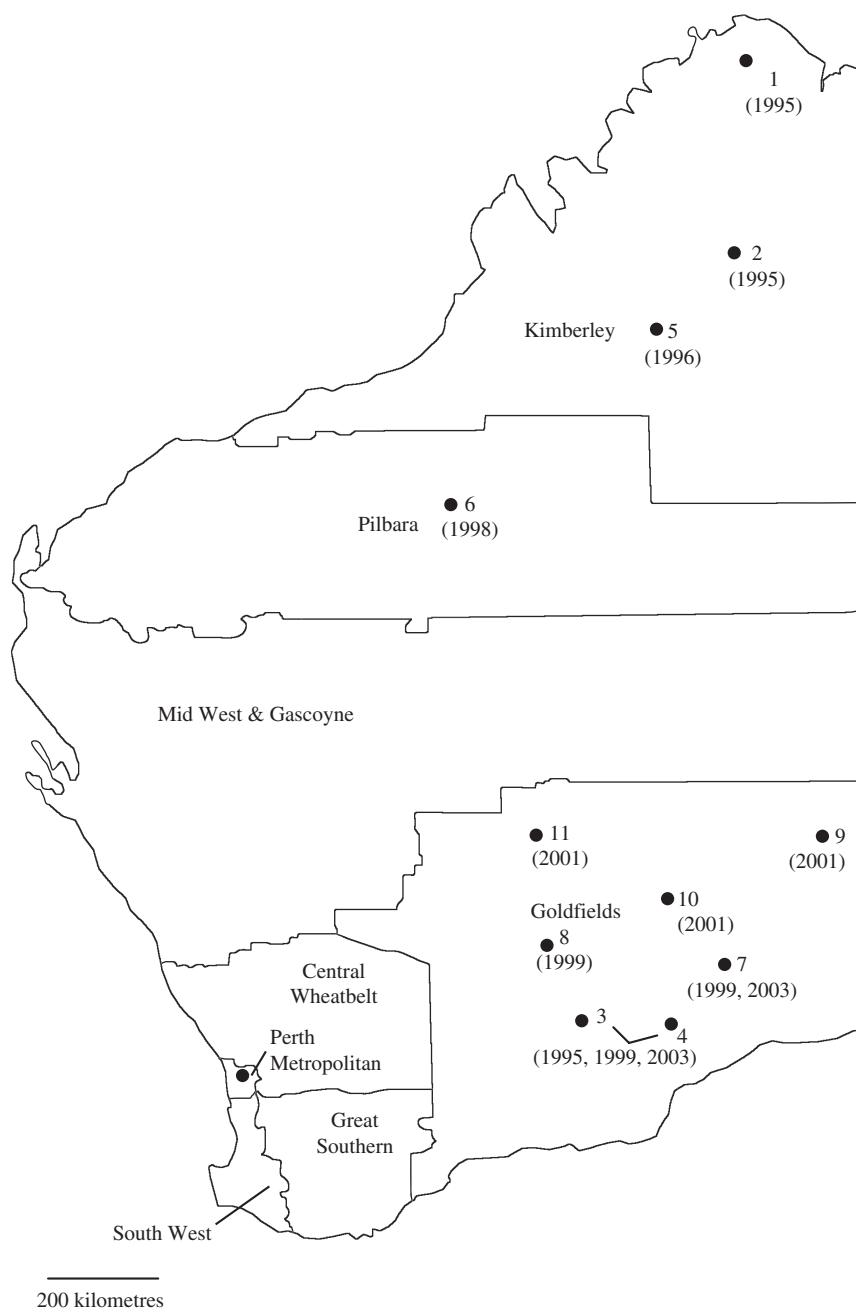
### Laboratory processing

Swabs were plated onto mannitol salt agar (MSA) (Oxoid, Basingstoke, UK) for detection of *S. aureus* and methicillin aztreonam mannitol salt agar (MAMSA)<sup>15</sup> or methicillin MSA (MMSA) (MSA containing 4  $\mu$ g of methicillin/mL) for detection of MRSA. All plates were incubated at 35°C. The MAMSA plates were read after 20 h incubation and the MSA and MMSA plates were read after 48 h incubation. Mannitol-fermenting colonies were cultured overnight in brain–heart infusion broth (Gibco Diagnostics, Gaithersburg, MD, USA) and identified as *S. aureus* by the tube coagulase test.

### Susceptibility testing

Antimicrobial susceptibility testing was performed by disc diffusion on Mueller–Hinton agar (MHA) (BBL, Becton Dickinson, Cockeysville, MD, USA) using Oxoid discs according to the method of the CLSI (formerly the NCCLS),<sup>16</sup> with fusidic acid susceptibility criteria as previously published.<sup>17</sup> All staphylococci were initially tested for methicillin resistance using a 1  $\mu$ g oxacillin disc (Oxoid, Basingstoke, UK). MRSA was confirmed by detection of the *mecA* and *nuc* genes in a multiplex PCR.<sup>18</sup> Following multilocus sequence typing (MLST) a representative MSSA from each sequence type (ST) was screened by PCR to confirm the absence of the *mecA* gene.<sup>19</sup>

For all MRSA an 18-antimicrobial antibiogram was performed using the following drugs: gentamicin, kanamycin, neomycin, streptomycin, erythromycin, lincomycin, chloramphenicol, minocycline, tetracycline, trimethoprim, sulfamethoxazole, fusidic acid, rifampicin, novobiocin, vancomycin, mupirocin, spectinomycin and ciprofloxacin. Erythromycin-inducible resistance to lincomycin was determined by the D-test.<sup>20</sup> For MSSA isolated after and including 1998 an eight-antimicrobial antibiogram was performed (erythromycin, tetracycline, trimethoprim, mupirocin, gentamicin,



**Figure 1.** Geographical regions of WA, locations of surveyed communities and years of screening. Geographical regions are named, communities are indicated numerically.

ciprofloxacin, rifampicin and fusidic acid). The 18-antimicrobial antibiogram and penicillin susceptibility testing were performed on representatives of all MSSA STs. All *S. aureus* that had an 18-antimicrobial antibiogram were tested for  $\beta$ -lactamase production using Nitrocefin discs according to the instructions of the manufacturer (BBL, Becton Dickinson, Franklin Lakes, NJ, USA). Resistograms were performed as previously described<sup>21</sup> on all MRSA, and all MSSA isolated after 1998.

#### *Contour-clamped homogeneous electric field electrophoresis*

Contour-clamped homogeneous electric field electrophoresis (CHEF) was performed as previously described<sup>22</sup> on all

isolates. Chromosomal banding patterns were scanned with a Fluor-S MultiImager and analysed by MultiAnalyst/PC (Bio-Rad Laboratories, Hercules, CA, USA) with a 0.8% band position tolerance. *S. aureus* isolates with  $\geq 80\%$  similarity were considered to belong to the same CHEF pulsotype; sub-pulsotypes were assigned according to the sub-clustering of patterns within the  $\geq 80\%$  similarity threshold. *S. aureus* NCTC8325 was used as the size standard. MRSA CHEF pattern pulsotypes were designated as previously published<sup>23</sup> and MSSA CHEF pattern pulsotypes were designated numerically. Isolates with pulsotypes containing fewer than three isolates were considered to be sporadic and, apart from PVL and antibiogram testing, were not investigated further in this study.

### MLST

MLST was performed as previously described.<sup>24</sup> All MRSA pulso-types and sub-pulso-types and all MSSA pulso-types and sub-pulso-types that contained three or more isolates were characterized by MLST. The sequences were submitted to <http://mlst.net> where an allelic profile was generated and an ST assigned. Clonal complex (CC) was determined using the eBURST V3 algorithm at the same website. Clones that diverged at no more than one of the seven MLST loci were considered to belong to the same CC. Double locus variants (dlvs) were included if the linking single locus variant (slv) was present in the MLST database. An *S. aureus* clone was defined by its ST. Isolates that belonged to the same CC were considered to be of the same genetic lineage.

### SCC mec typing

SCCmec typing was performed using previously published primers that identified the class of *mec* complex and type of cassette chromosome recombinase (*ccr*) complex encoded on the element.<sup>25</sup> Structural architecture was determined with the multiplex PCR primers of Zhang *et al.*<sup>26</sup> and extra primers were utilized to test for SCCmec type IV subtypes a, b, c and d.<sup>27</sup> SCCmec nomenclature was as proposed by the International Working Group on the Classification of Staphylococcal Cassette Chromosome (IWG-SCC) Elements. Briefly, the structural type is indicated by Roman numerals with a lower case Arabic letter indicating the sub-type and the *ccr* and *mec* complexes are indicated by an Arabic number and letter, respectively, in parentheses.

### PVL

The PVL determinant was detected using previously published primers<sup>28</sup> and confirmed by sequencing.

### Accessory genetic regulator (*agr*)

*agr* was typed using either the ArrayTube System according to the manufacturer's instructions (Clondiag, Jena, Germany) or primers from previous studies.<sup>29,30</sup>

### Arginine catabolic mobile element (ACME)

The ACME was detected by PCR as described previously.<sup>31</sup>

### Criteria for testing

A colony was selected from each plate from each anatomical site. If there was more than one morphological colony type a representative of each was tested. If these isolates were subsequently found to be different by the typing methods they were included in the study as individual strains.

## Results

Of the 2146 sets of screening swabs, 663 sets grew MSSA and 153 grew MRSA. Table 1 presents the sites that were positive for MSSA or MRSA for each set of screening swabs. Of the 1172 *S. aureus* isolated, 933 were MSSA and 239 were MRSA. Overall, 762 isolates of *S. aureus* consisting of 523 MSSA (from 454 sets of screening swabs) and 239 MRSA (from 153 sets of screening swabs) were characterized in this study.

**Table 1.** Sites of isolation of MSSA and MRSA from people in remote WA communities

Site of isolation	MSSA	MRSA
Anterior nares only	139	49
Anterior nares and throat	56	15
Anterior nares and skin lesions	33	9
Anterior nares, throat and skin lesions	21	6
Throat only	163	30
Throat and skin lesions	43	5
Skin lesions only	208	39
Total positive screening sets	663	153

There was a variation in the ratio of MSSA and MRSA carriage between the three geographical regions. MRSA comprised 4%, 24% and 32% of total *S. aureus* from the Kimberley, Pilbara and Goldfields regions, respectively.

There were differences in the colonization sites of MSSA and MRSA. MSSA was grown from 249 (37.6%), 283 (42.7%) and 305 (46%) anterior nares, throat and skin lesion swabs, respectively, while 79 (51.6%), 56 (36.6%) and 59 (38.6%) anterior nares, throat and skin lesion swabs, respectively, grew MRSA. For MSSA the highest rates of colonization were from skin lesions followed by throat and for MRSA they were anterior nares followed by skin lesions. When considering the overall positive screening sites, the highest recovery of MSSA per screening set was from throat and/or skin lesion swabs (79%) while the highest recovery of MRSA was from anterior nares and/or skin lesion swabs (80.4%). Of 59 sets of screening swabs in which MRSA was cultured from skin lesions, 15 (25%) demonstrated co-colonization with MRSA in the anterior nares.

### Genetic lineages

Using CHEF the 523 MSSA were classified into 84 pulso-types, of which 27 pulso-types had three or more isolates (Table 2). The 239 MRSA were classified into five pulso-types (Table 3). These five pulso-types also contained MSSA, with MRSA pulso-types WA-1, -2, -3, -4 and -5 corresponding to MSSA pulso-types MSSA1, 5, 3, 14 and 26, respectively (Tables 2 and 3). Overall 92.7% (467 MSSA and 239 MRSA) of the 762 *S. aureus* clustered into 27 pulso-types, from which 21 STs belonging to 13 CCs and two Singleton lineages were identified by MLST (Table 4). Eight lineages (CC15, CC121, CC101, CC25, CC20, CC398, CC12 and CC188) and the two Singleton lineages (Singleton 93 and Singleton 760) contained MSSA only. Five lineages (CC1, CC5, CC88, CC45 and CC8) contained MSSA and MRSA. CC5 contained two MRSA clones, ST5-MRSA-IVa (2B) and ST73-MRSA-IVa (2B) (Figure 2) and CC45 contained two MRSA clones, ST45-MRSA-V (5C2) and ST45-MRSA-IVa (2B).

Seven previously undescribed STs were identified: Singleton ST760-MSSA; the CC1 clones ST761-MSSA and ST762-MSSA; the CC5 clone ST73-MSSA; the CC15 clone ST832-MSSA; the CC398 clone ST813-MSSA; and ST833-MSSA from CC188. Together with the previously reported ST73-MRSA-IVa (2B)<sup>32</sup> and ST93-MRSA-IVa (2B), which are rarely found outside



**Table 2.** Characteristics of representative methicillin-susceptible *S. aureus* from remote WA communities

Isolate	Resistance/Bla	Pulsotype	CC	ST, allelic profile	PVL	agr type
W17S	PCd Bla <sup>+</sup>	MSSA6	S	93, 6-64-44-2-43-55-51	+	III
N126S	PEL <sup>I</sup> Cd Bla <sup>+</sup>	MSSA6	S	93, 6-64-44-2-43-55-51	+	III
C229T	Cd Bla <sup>+</sup>	MSSA6	S	93, 6-64-44-2-43-55-51	+	III
W20S	s Bla <sup>-</sup>	MSSA2	15	15, 13-13-1-1-12-11-13	-	II
WL90T	PEL <sup>I</sup> Cd Bla <sup>+</sup>	MSSA25	15	15, 13-13-1-1-12-11-13	-	II
N133T	PCCd Bla <sup>+</sup>	MSSA29	15	15, 13-13-1-1-12-11-13	-	II
W16S	PCd Bla <sup>+</sup>	MSSA25	15	15, 13-13-1-1-12-11-13	-	II
P3S	PECd Bla <sup>+</sup>	MSSA20	15	15, 13-13-1-1-12-11-13	-	II
J27T	PCd Bla <sup>+</sup>	MSSA30	15	15, 13-13-1-1-12-11-13	-	II
K43T	PCd Bla <sup>+</sup>	MSSA22	15	832, 13-13-111-1-12-11-13	-	II
WL6N	PCd Bla <sup>+</sup>	MSSA27	5	5, 1-4-1-4-12-1-10	-	II
K185N	PCd Bla <sup>+</sup>	MSSA3	5	73, 1-4-27-4-12-1-10	-	II
K153N	PCdEb Bla <sup>+</sup>	MSSA3	5	73, 1-4-27-4-12-1-10	-	II
WL36N	P Bla <sup>+</sup>	MSSA3	5	73, 1-4-27-4-12-1-10	-	II
K112L	PCd Bla <sup>+</sup>	MSSA12	5	6, 12-4-1-4-12-1-3	-	I
Y15S	PFCd Bla <sup>+</sup>	MSSA1a	1	1, 1-1-1-1-1-1-1	-	III
Y74T	PCd Bla <sup>+</sup>	MSSA1b	1	761, 1-1-104-1-1-103-1	-	NT
K45S	PECdHg Bla <sup>+</sup>	MSSA1c	1	762, 1-1-104-1-1-1-1	-	III
K120L	PCd Bla <sup>+</sup>	MSSA1c	1	762, 1-1-104-1-1-1-1	-	III
C38S	PTCd Bla <sup>+</sup>	MSSA11	S	760, 10-1-1-1-1-102-1	-	III
C49N	PCd Bla <sup>+</sup>	MSSA14	45	45, 10-14-8-6-10-3-2	-	I
C54N	s Bla <sup>-</sup>	MSSA14	45	45, 10-14-8-6-10-3-2	-	I
C30S	P Bla <sup>+</sup>	MSSA21	45	45, 10-14-8-6-10-3-2	-	IV
M11N	PCd Bla <sup>+</sup>	MSSA17	45	45, 10-14-8-6-10-3-2	-	I
K102N	PCd Bla <sup>+</sup>	MSSA28	45	508, 10-40-8-6-10-3-2	-	I
K25S	PCd Bla <sup>+</sup>	MSSA7	121	121, 6-5-6-2-7-14-5	+	IV
Y1S	P Bla <sup>+</sup>	MSSA23	121	121, 6-5-6-2-7-14-5	-	IV
WB94E	P Bla <sup>+</sup>	MSSA19	121	121, 6-5-6-2-7-14-5	-	IV
W67N	PCd Bla <sup>+</sup>	MSSA5	88	78, 22-1-14-23-12-53-31	-	III
W91T	PCd Bla <sup>+</sup>	MSSA4	101	101, 3-1-14-15-11-19-3	-	I
W11T	PCd Bla <sup>+</sup>	MSSA33	25	25, 4-1-4-1-5-5-4	-	I
C57S	PCd Bla <sup>+</sup>	MSSA13	20	20, 4-9-1-8-1-10-8	-	I
J107N	PCd Bla <sup>+</sup>	MSSA24	20	20, 4-9-1-8-1-10-8	-	I
N91T	P Bla <sup>+</sup>	MSSA26	8	8, 3-3-1-1-4-4-3	-	I
W101S	s Bla <sup>-</sup>	MSSA10	329	813, 3-37-19-2-20-26-32	-	I
C33S	PCd Bla <sup>+</sup>	MSSA12	12	12, 1-3-1-8-11-5-11	-	II
W36S	PCd Bla <sup>+</sup>	MSSA8	188	833, 100-1-1-8-12-1-1	-	III

Bla,  $\beta$ -lactamase; C, chloramphenicol; Cd, cadmium acetate; E, erythromycin; Eb, ethidium bromide; F, Fusidic acid; Hg, mercuric chloride; L, lincomycin; P, penicillin; T, tetracycline; superscript I, inducible; superscript +, positive; superscript -, negative; s, susceptible to all antimicrobials tested; S, singleton lineage; NT, non-typeable.

Australia, these isolates appear to represent geographically limited clones that have probably emerged in Australia.

The lineages of *S. aureus* that contained the most isolates were CC1 (18%), CC5 (17.5%), Singleton 93 (14.7%), CC15 (10.6%), CC45 (8.4%), CC88 (6.4%) and CC121 (4.6%). There was no evidence of the emergence of new dominant clones of *S. aureus* during the period of the surveys.

**MSSA.** The most prevalent MSSA were ST93-MSSA from the Singleton 93 lineage (21.4%), ST15-MSSA from CC15 (14.9%), ST73-MSSA from CC5 (10.1%) and ST45-MSSA from CC45 (5.9%) (Table 4).

Four lineages of MSSA (CC1, CC5, CC15 and CC45) contained slvs, with CC1 also containing a dlvs (ST761-MSSA) (Table 2). ST760-MSSA was an ST1-MSSA dlvs; however,

because the linking allele could not be found in the MLST database it was classified as a new Singleton lineage.

Some of the MSSA lineages showed divergence of CHEF pattern pulsotypes (Table 2). CC15 had diversified into five unrelated pulsotypes, of which one was the slvs ST832-MSSA. CC5 contained three pulsotypes representing each of the CC5 clones, ST73-MSSA, ST5-MSSA and ST6-MSSA. There were four unrelated pulsotypes in CC45, one of them being the slvs ST508-MSSA clone. CC121 had three pulsotypes, and CC20 had two. The remaining lineages each had one CHEF pulsotype. CC1 isolates also belonged to only one pulsotype; however, there were three sub-pulsotypes that represented the clones ST1-MSSA, ST761-MSSA and ST762-MSSA. ST6-MSSA (CC5) and ST12-MSSA (CC12), although genetically unrelated by MLST, both had the same MSSA12 pulsotype.

## Genetics of *S. aureus* in remote communities

**Table 3.** Characteristics of representative MRSA from remote WA communities

Isolate	Resistance and Bla	Pulsotype	CC	ST, allelic profile	SCCmec	PVL	agr type	ACME
WBG8287	EL <sup>I</sup> FCd Bla <sup>+</sup>	WA-1	1	1, 1-1-1-1-1-1-1	IVa (2B)	—	III	—
WBG8375	EL <sup>I</sup> Cd Bla <sup>+</sup>	WA-1a	1	1, 1-1-1-1-1-1-1	IVa (2B)	—	III	—
WBG9409	EL <sup>I</sup> FCd Bla <sup>+</sup>	WA-1c	1	1, 1-1-1-1-1-1-1	IVa (2B)	—	III	—
WBG8361	EL <sup>I</sup> Cd Bla <sup>+</sup>	WA-1d	1	1, 1-1-1-1-1-1-1	IVa (2B)	—	III	—
M28S	Cd Bla <sup>+</sup>	WA-1f	1	1, 1-1-1-1-1-1-1	IVa (2B)	—	III	—
WBG8366	EL <sup>I</sup> Bla <sup>+</sup>	WA-2	88	78, 22-1-14-23-12-53-31	IVa (2B)	—	III	—
WL106N	EL Bla <sup>+</sup>	WA-2a	88	78, 22-1-14-23-12-53-31	IVa (2B)	—	III	—
C219N	Cd Bla <sup>+</sup>	WA-2c	88	78, 22-1-14-23-12-53-31	IVa (2B)	—	III	—
C8N	EL <sup>I</sup> Cd Bla <sup>+</sup>	WA-3	5	5, 1-4-1-4-12-1-10	IVa (2B)	—	II	—
WBG8381	s Bla <sup>—</sup>	WA-3a	5	5, 1-4-1-4-12-1-10	IVa (2B)	—	II	—
WB43S	EL <sup>I</sup> Bla <sup>+</sup>	WA-3b	5	73, 1-27-1-4-12-1-10	IVa (2B)	—	NT	—
WL36N	Cd Bla <sup>—</sup>	WA-3b	5	73, 1-27-1-4-12-1-10	IVa (2B)	—	II	—
WBG8379	EL <sup>I</sup> Cd Bla <sup>+</sup>	WA-3c	5	5, 1-4-1-4-12-1-10	IVa (2B)	—	II	—
WB101N	EL <sup>I</sup> Bla <sup>+</sup>	WA-3h	5	5, 1-4-1-4-12-1-10	IVa (2B)	—	II	—
WBG8404	CdAs Bla <sup>+</sup>	WA-4	45	45, 10-14-8-6-10-3-2 45	V (5C2)	—	Ia	—
WBG8399	CdAs Bla <sup>+</sup>	WA-4a	45	45, 10-14-8-6-10-3-2 45	V (5C2)	—	I	—
WBG8355	CdAs Bla <sup>+</sup>	WA-4b	45	45, 10-14-8-6-10-3-2 45	IVa (2B)	—	I	—
WBG7583	EL <sup>I</sup> TCd Bla <sup>+</sup>	WA-5	8	8, 3-3-1-1-4-4-3	IVa (2B)	—	I	—

As, sodium arsenate; Bla,  $\beta$ -lactamase; Cd, cadmium acetate; E, erythromycin; F, fusidic acid; L, lincomycin; superscript I, inducible; superscript +, positive; superscript —, negative; s, susceptible to all antimicrobials tested; NT, non-typeable.

**Table 4.** Genetic lineages of *S. aureus* present in remote WA communities

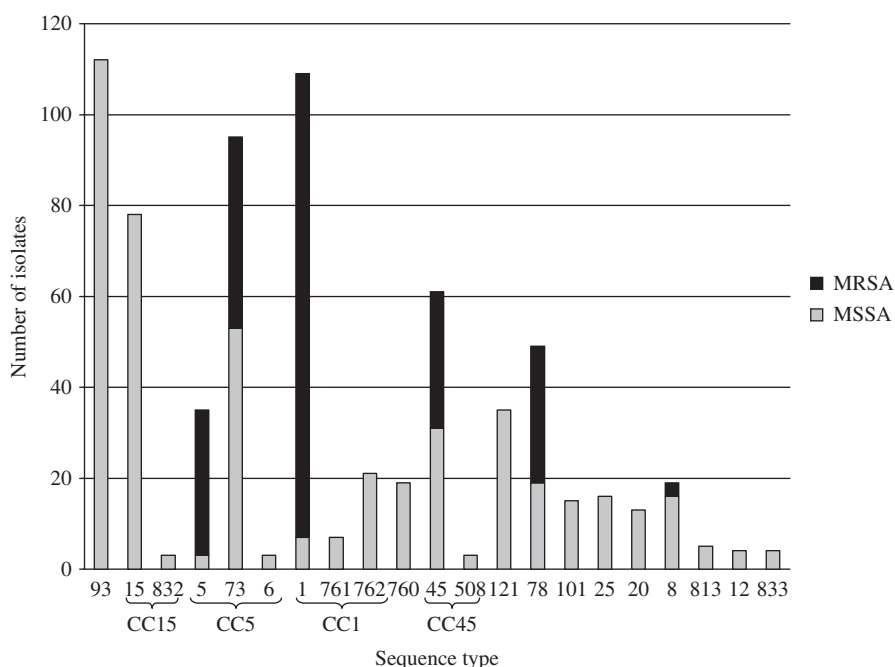
Genetic lineage, CC and ST	MRSA number (%)	MSSA number (%)
Singleton (93) and 93		112 (21.4)
15 and 15		78 (14.9)
15 and 832		3 (0.6)
5 and 5	32 (13.4)	3 (0.6)
5 and 73	42 (17.6)	53 (10.1)
5 and 6		3 (0.6)
1 and 1	102 (42.7)	7 (1.3)
1 and 761		7 (1.3)
1 and 762		21 (4)
Singleton (760) and 760		19 (3.6)
45 and 45	30 (12.5)	31 (5.9)
45 and 508		3 (0.6)
121 and 121		35 (6.7)
88 and 78	30 (12.5)	19 (3.6)
101 and 101		15 (2.8)
25 and 25		16 (3.1)
20 and 20		13 (2.5)
8 and 8	3 (1.3)	16 (3.1)
398 and 813		5 (1)
12 and 12		4 (0.8)
188 and 833		4 (0.8)
Sporadic		56 (10.7)
Totals	239 (100)	523 (100)

MRSA. When compared with MSSA there was less diversity in the MRSA lineages. Five lineages were identified in the communities screened in the 1995 surveys. No additional lineages were found in these or the other communities in subsequent surveys. ST1-MRSA-IVa (2B) was the most frequently isolated MRSA clone (42.7%), followed by ST73-MRSA-IVa (2B) (17.6%), ST5-MRSA-IVa (2B) (13.4%), ST45-MRSA-V (5C2) and ST45-MRSA-IVa (2B) (12.5%), ST78-MRSA-IVa (2B) (12.5%) and ST8-MRSA-IVa (2B) (1.3%) (Tables 3 and 4).

The five MRSA lineages corresponded to five CHEF pulsotypes that have previously been identified.<sup>11,33</sup> Four of the pulsotypes had sub-pulsotypes. Although all lineages containing MRSA also contained MSSA, they were not the largest MSSA lineages (Table 4). The CC1 MRSA clone, ST1-MRSA-IVa (2B), formed 42.7% of all MRSA while the methicillin-susceptible counterpart, ST1-MSSA, formed only 1.3% of the MSSA population. Similarly, in CC5, ST5-MRSA-IVa (2B) was 13.4% of the MRSA while ST5-MSSA formed only 0.6% of the MSSAs and ST78-MRSA-IVa (2B) was 12.5% of the MRSA while the corresponding MSSA was only 3.6% of the MSSA population. MRSA were not found in the largest MSSA lineages of Singleton 93 and CC15.

### Antimicrobial resistance

MSSA. A full 18-antimicrobial antibiogram and penicillin susceptibility testing was performed on 37 MSSA clones representative of the lineages. All were resistant to penicillin and produced  $\beta$ -lactamase except for three, which were fully susceptible (Table 2). Two isolates expressed an ML<sub>S</sub><sub>Bi</sub> resistance phenotype (inducible resistance to erythromycin and erythromycin-inducible resistance to lincomycin), an additional



**Figure 2.** Distribution of MSSA and MRSA amongst the genetic lineages of *S. aureus* present in remote WA communities. Brackets indicate clones that belong to the same CCs.

isolate had constitutive erythromycin resistance and another was chloramphenicol resistant.

An eight-antimicrobial antibiogram was performed on 363 isolates. Eighty-three (22.9%) were fully susceptible to all antimicrobials; 134 (36.9%) were erythromycin resistant, six (1.7%) were fusidic acid resistant, 4 (1.1%) were gentamicin resistant, 6 (1.7%) were trimethoprim resistant and 9 (2.5%) were tetracycline resistant.

A resistogram was performed on 423 MSSA. Of these, 254 (60%) were cadmium resistant, 4 were arsenate resistant, 1 was mercuric chloride resistant, 1 was mercuric chloride and phenyl mercuric acetate resistant, and 4 were ethidium bromide resistant. There were no associations between antimicrobial resistance profile and genetic lineage.

**MRSA.** All except two of the 239 MRSA isolates were additionally resistant to fewer than two antibiotic classes and therefore non-multi-resistant.<sup>10</sup> Fifty-nine (24.7%) of the isolates were fusidic acid resistant, all of which were ST1-MRSA-IVa (2B). Within CC1, 59 (57.8%) of the 102 MRSA isolates were fusidic acid resistant. One hundred and thirty three isolates (55.6%), including isolates from ST1-MRSA-IVa (2B), ST78-MRSA-IVa (2B), ST5-MRSA-IVa (2B) and ST73-MRSA-IVa (2B), were erythromycin resistant, four (1.7%) isolates, two from ST5-MRSA-IVa (2B) and one each from ST45-MRSA-V (5C2) and ST8-MRSA-IVa (2B), were mupirocin resistant and three (1.3%), two from ST8-MRSA-IVa (2B) and one ST1-MRSA-IVa (2B) were tetracycline resistant. All except two of the representative MRSA (WBG8381 and WL36N) produced  $\beta$ -lactamase (Table 3).

The most prevalent MRSA lineage was also resistant to the most antibiotics. Forty-two of the ST1-MRSA-IVa (2B) isolates that were fusidic acid resistant also expressed the  $MLS_{Bi}$  resistance phenotype; one of these was additionally tetracycline resistant and therefore was multi-resistant by definition.<sup>10</sup> The other

multi-resistant isolate was an ST45-MRSA-V (5C2) skin lesion isolate that had the  $MLS_{Bi}$  resistance phenotype as well as being gentamicin, kanamycin and mupirocin resistant. Interestingly, the individual who harboured this clone also harboured ST45-MRSA-V (5C2) isolates from the anterior nares and throat that were susceptible to all antibiotics except the  $\beta$ -lactams.

All except 10 of the MRSA (95.8%) were cadmium resistant and 26 (10.9%) were arsenate resistant. Arsenate resistance was exclusively linked with ST45-MRSA-V (5C2) and ST45-MRSA-IVa (2B) clones.

### PVL

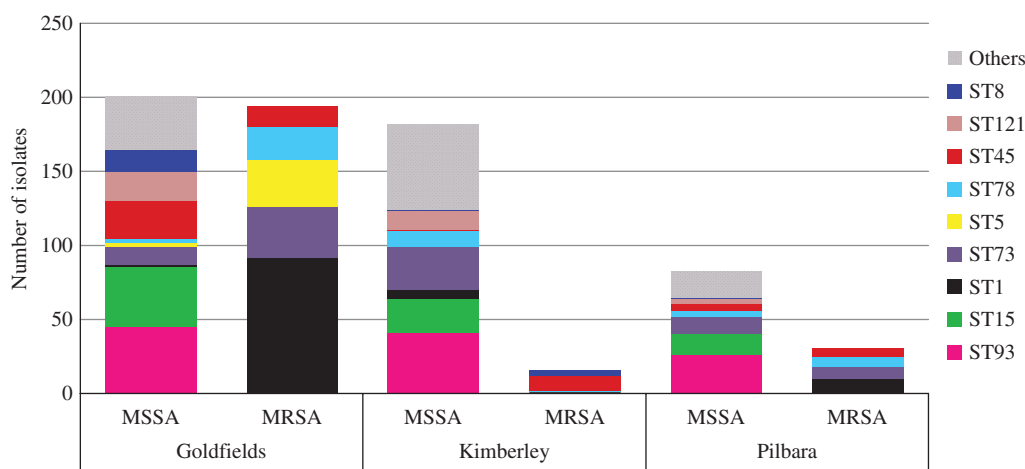
At least one representative isolate from all MSSA and MRSA CHEF pulsotypes and sub-pulsotypes was tested for the presence of the PVL determinant. Two lineages of MSSA harboured the determinant; seven of eight ST93-MSSA tested were found to carry PVL and of three ST121-MSSA tested one carried the determinant (Table 2). No MRSA carried PVL.

### agr and ACME

The *agr* type was determined on representative clones and revealed four major *agr* types in the *S. aureus* isolates (Tables 3 and 4). Five lineages (CC101, CC25, CC20, CC329 and CC8) were *agr* I, two (CC15 and CC12) were *agr* II, five (Singleton 93, CC1, Singleton 760, CC88 and CC188) were *agr* III, and one lineage (CC121) was type IV. CC5 isolates were *agr* II except for a ST6-MSSA clone that was type I. CC45 had members in *agr* types I, Ia and IV. Two isolates (WB43S and Y74T) were non-typeable.

MSSA clones from all *agr* groups were present; however, no *agr* type IV MRSA was found. The PVL-positive clones ST93-MSSA and ST121-MSSA belonged to *agr* groups III and

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**Figure 3.** Distribution of the predominant genetic lineages of *S. aureus* throughout remote communities in three geographical regions of WA.

IV, respectively. There was no correlation between site of colonization and *agr* type, and clones with both the same and different *agr* types colonized the same individual and/or sites (not shown).

Representative MRSA were tested for the presence of the ACME and none encoded the element.

### Geographical distribution

There were local differences in the *S. aureus* clones present in the geographical regions (Figure 3). ST1 was the predominant clone in the Goldfields region and ST93 and ST73 were predominant in Kimberley and Pilbara. Thirteen of the 21 clones (STs 1, 6, 8, 15, 20, 25, 45, 73, 78, 93, 121, 760 and 762) were found in all geographical regions.

The MRSA clones ST1-MRSA-IVa (2B), ST78-MRSA-IVa (2B) and ST45-MRSA-V (5C2) were found in all regions, ST5-MRSA-IVa (2B) and ST45-MRSA-IVa (2B) were found only in the Goldfields, and ST8-MRSA-IVa (2B) was found only in the Kimberley region. The most prevalent MRSA clone in the Goldfields was ST1-MRSA-IVa (2B) (47%) followed by ST73-MRSA-IVa (2B) (18%). Similarly, in the Pilbara region the prevalent MRSA clones were ST1-MRSA-IVa (2B) (32%) and ST73-MRSA-IVa (2B) (26%), while in Kimberley the predominant clone was ST45-MRSA-V (5C2) (62.5%).

Clones ST1-MRSA-IVa (2B), ST78-MRSA-IVa (2B), ST5-MRSA-IVa (2B) and ST73-MRSA-IVa (2B) were found in all years of the surveys and were considered to be endemic in the communities. The ST8-MRSA-IVa (2B), ST45-MRSA-V (5C2) and ST45-MRSA-IVa (2B) clones were not found subsequent to 1998.

### Genetics of colonization

There was no apparent correlation between ST and site of isolation (not shown).

People harboured clones belonging to a diversity of genetic lineages at the same or multiple sites. MRSA and MSSA were found together in 79 sets of screening swabs; all clones from 30 of these were characterized. No person was found to have MRSA and MSSA of the same genetic lineage at either the same or different sites. Two hundred and fifty-seven (12%) sets

of screening swabs had MSSA at more than one site. Of 56 with characterized clones at multiple sites, only 12 (21%) had clones of the same genetic lineage at all sites and 44 (79%) had a different lineage at each site. Two sets of screening swabs that yielded MSSA at three sites had isolates of a different genetic lineage at each site.

In contrast to MSSA, of 39 sets of screening swabs where MRSA was found at multiple sites, 36 (92%) had clones of the same genetic lineage at all sites with only three (8%) harbouring MRSA of different lineages.

## Discussion

Population studies of *S. aureus* thus far have identified five main genotypic clusters, CC5, CC8, CC22, CC30 and CC45, as forming the essential genetic backgrounds of *S. aureus*, with differences occurring principally in the local prevalence of the genotypes and the presence of minor clones.<sup>34–36</sup> Although these studies have been from Europe and the USA, a study by Melles *et al.*<sup>34</sup> performed in Indonesia, which has prehistoric links with remote WA, reported a similar *S. aureus* population structure to that of Europe and the USA. This study, however, reveals that the population structure of *S. aureus* in the geographically remote regions of WA is different. This difference is probably a consequence of the geographic and cultural isolation of the remote populations of WA; however, it has had an important influence on the epidemiology of MRSA in the entire WA community.

From a genetically diverse background consisting essentially of 21 clones of *S. aureus*, seven clones of MRSA belonging to five CCs were found. Four of the clones were considered to have been endemic in the communities and have subsequently become the most prevalent CA-MRSA clones in the wider WA community.<sup>23</sup> State-wide surveillance has revealed that in December 2006, ST1-MRSA-IVa (2B), ST78-MRSA-IVa (2B) and the CC5 clones [ST73-MRSA-IVa (2B) and ST5-MRSA-IVa (2B)] comprised 56.7%, 30.5% and 8.9% of clinical and surveillance CA-MRSA in WA, respectively. The CC45 clones [ST45-MRSA-V (5C2) and ST45-MRSA-IVa (2B)], and ST8-MRSA-IVa (2B), which were not found in the remote communities after 1998, formed only 1.9% and 0.8%, respectively, of



clinical and surveillance CA-MRSA in WA in 2006<sup>11</sup> suggesting that they are not as well adapted to the WA community environment.

The MRSA did not belong to the most prevalent MSSA lineages, yet, with the exception of ST8-MRSA-IVa (2B), they formed the greater proportion of isolates present in the lineage to which they belonged, suggesting that an advantage was gained by acquisition of the SCCmec element. It would appear, however, that only a limited number of clones acquired and maintained the SCCmec element, even though  $\beta$ -lactamase-stable  $\beta$ -lactams were widely used empirically in the communities. The clonal structure of MRSA and the small amount of genetic diversity when compared with MSSA indicate not only the more recent emergence of MRSA, but also that dissemination of MRSA has probably occurred along clonal lines by well-adapted community clones that could support the SCCmec element.

The most prevalent MSSA lineage was the PVL-positive Singleton 93 clone ST93-MSSA, which has been rarely found outside Australia. No Singleton 93 MRSA was found during the period of the surveys. PVL-positive ST93-MRSA-IVa (2B), also known as the Queensland clone, however, is an important Australian CA-MRSA that was originally found in a Caucasian population in Queensland in 2000 and has been reported in Indigenous people from Queensland<sup>5,37</sup> and the Northern Territory.<sup>38</sup> It is interesting that in an environment of high  $\beta$ -lactam use a methicillin-resistant variant of ST93-MSSA was not found in WA during these surveys.

*S. aureus* isolates from most of the lineages were found at all sites tested. The highest recovery of MSSA of 42.6% was from the throat while for MRSA the highest recovery of 51.6% was from the anterior nares. Although the anterior nares is the preferred screening site for population studies, in this study many isolates of *S. aureus* would have been missed if the throat and skin lesions had not also been swabbed. It has been established previously that there is a high incidence of skin pathology associated with *S. aureus* in remote Australian communities<sup>38</sup> and the recovery figures in this study are clearly influenced by the high numbers of skin lesions found amongst the survey participants.

The clonal nature of MRSA and the tendency for people carrying MRSA at multiple sites to harbour clones of the same genetic lineage as opposed to those with MSSA, who tended to have different lineages at each site, indicates that MRSA in the WA remote communities are well-adapted colonizers that could possibly displace MSSA as asymptomatic commensal organisms. Furthermore, unless the use of  $\beta$ -lactamase-stable antibiotics is curtailed they could become the predominant colonizing organisms in the communities.

Very few remote region *S. aureus* isolates were resistant to multiple antimicrobials; however, the potential for the emergence of resistance was indicated by the presence of several antimicrobial resistance determinants amongst the population. In addition to the SCCmec element, determinants for resistance to penicillin, fusidic acid, MLS<sub>Bi</sub>, erythromycin, tetracycline, gentamicin, kanamycin, mupirocin, trimethoprim and chloramphenicol were present.

The resistance determinants for  $\beta$ -lactamase production, MLS<sub>Bi</sub>, mupirocin and trimethoprim in remote WA community MRSA are plasmid borne,<sup>33,39</sup> and other studies have shown that those for gentamicin and kanamycin are on a transposon while those for erythromycin, tetracycline and chloramphenicol are on

plasmids. In view of the increased isolation rates of CA-MRSA in clinical specimens in WA it would be instructive to assess the current status of CA-MRSA in the remote communities to determine if there is a need to control the local use of antibiotics. Such control could be predicated upon the known resistance determinants in the *S. aureus* populations of the communities. It is imperative that careful antibiotic management guidelines are established and administered in the communities to prevent CA-MRSA acquiring additional resistance determinants and spreading further. The importance of this was indicated from results (not shown) from communities 4 and 5. When these communities were initially screened the prevalence of MRSA was 43% and 22%, respectively. As a consequence, non- $\beta$ -lactam antibiotics replaced the empirical administration of  $\beta$ -lactam antibiotics for *S. aureus* infections and re-screening of the communities four years later revealed that the prevalence of MRSA had dropped to 11% and 7%, respectively.

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## Transparency declarations

None to declare.

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